Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement
This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (https://scholarworks.iu.edu/dspace/handle/2022/7911).

Adrenocorticotropic Hormone (ACTH) Challenge Protocol

ACTH solution recipe

Vials of ACTH powder are sold by the IU rather than by weight, although it is typically ~80-95 IU/mg.

To reconstitute a 100 I.U. vial of ACTH for injection.

Ideal dosage is 100 IU/kg. Assuming the average junco is ~20 g, and the injection size is 50 µL:

$$\frac{20\ \text{g}}{\text{bird}} \times \frac{1\ \text{kg}}{1000\text{g}} \times \frac{100\ \text{IU}}{\text{kg}} = \frac{2\ \text{IU}}{\text{bird}}$$

$$\frac{2\ \text{IU}}{50\ \mu\text{L}} \times \frac{1\ \text{vial}}{100\ \text{IU}} \times \frac{1000\ \mu\text{L}}{1\ \text{mL}} = \frac{1\ \text{vial}}{2.5\ \text{mL solvent}}$$

Solvent can be PBS or Lactated Ringer's Solution.

Aliquot solution into 10 x 250 µL vial (enough for 5 injections per aliquot, or charging the syringe + 1-2 injections).

NOTE: ACTH is both temperature and light sensitive, so keep aliquots frozen until use, and wrapped in foil as much as possible.

ACTH challenge (modified from GnRH challenge protocol)

Charging the syringe:

1. Thaw a vial of ACTH.
2. Prepare a syringe as follows:
    - Using a disposable 1 cc syringe with needle attached draw up 0.2 cc of ACTH solution.
    - Hold the disposable syringe with the needle pointing upward and draw in 0.2 cc of air.
    - Flick the syringe with your finger to get the air bubbles to the top. [note: helps to flick hard below where the liquid is.]
    - With the needle still pointing upward, eject the air until a drop of liquid appears at the tip of the needle.
    - Take the plunger out of the 50 µl Hamilton syringe (glass-tipped, the kind to which you

add a needle).

• Make sure there is no liquid in the barrel of the Hamilton syringe. Use the disposable syringe to fill 1/3 of the Hamilton syringe from the back end. (make sure there are NO air bubbles). [It helps to hold the Hamilton in your left hand with the back end slightly tilted downwards, and use your right hand to place the tip of the needle of the disposable syringe into the barrel of the Hamilton bevel side up.]

• Use the plunger of the Hamilton syringe to push the solution through the Hamilton syringe and eject the ACTH solution back into the vial. Now there should no longer be an air space at the needle end of the syringe.

• Now put the glass tip of the Hamilton syringe directly into the vial (do not attach a needle yet) and fill the syringe with fluid (there should be no air bubbles).

• Put a clean 26 g 1/2 inch needle on the Hamilton syringe and eject its contents through the needle tip back into the vial.

• Eject the rest of the ACTH solution out of the disposable syringe back into the vial.

Challenge Protocol:

1. Start a timer when you first disturb the bird.
2. Once the bird is in hand, take a pre-challenge blood sample (~50 µL) from the alar wing vein. Note the time (on the timer) that you start and finish the blood draw.
3. Draw up 50 µL of ACTH solution from primed Hamilton syringe.
4. Use an alcohol swab to wipe the feathers away from the pectoral muscle of the bird.
5. Using your thumb pull the skin to one side, insert 1/3 - 1/2 of the length of the needle at a shallow angle into the pectoral muscle approximately 2-3 mm lateral to the keel of the sternum, inject the ACTH, remove the needle, and let the skin slide back in place. **Record the time of injection.** [*If the solution leaks back or the bird bleeds at the injection site, make a note.]*
6. Hold the bird in an opaque bag for 28-29 minutes. At 30 minutes from the time of injection, take a second blood sample (~50 µL) from the alar wing vein. Note the time that the blood draw was started and finished.
7. Repeat step 6, taking a third blood sample at 60 minutes post-injection.
8. If you are injecting many birds, wipe the needle with an alcohol swab between injections, but use the same needle to avoid having to evacuate air bubbles from new needles before every injection.
9. Once finished, discard the needle and rinse the Hamilton syringe several times with distilled water.